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Rapid growth at lower temperatures by the larvae of *Celastrina sugitanii* (Lepidoptera, Lycaenidae)*

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Abstract *Celastrina sugitanii* in the San-in District of Japan is a specialist depending on flowers of *Aesculus turbinata*. A field survey carried out in Misasa, Tottori Prefecture during 2003–2005 indicated that they laid eggs in late April. In late May the mature larvae left the host tree and fell to the ground surface, as was demonstrated by more than a hundred larvae collected with litter-fall traps under the host tree. The period for egg and larval stages in the field was thus estimated to be *ca* 36 days. Rearing tests under different temperature conditions (18, 21, 24, 27°C; all 16L8D) in the laboratory revealed an extraordinary developmental zero point of the larvae, -0.9°C, despite of a normal value of 7.8°C for the congeneric *C. argiolus*. The extreme value, caused by rapid larval growth at lower temperatures, should imply a climatic adaptation developed in *C. sugitanii* which needs to accomplish almost all its food assimilation within a restricted flowering period of *Aesculus turbinata* in May.

Key words *Celastrina sugitanii*, developmental zero point, climatic adaptation, life cycle synchronization.

Introduction

Almost all *Celastrina* butterflies depend on flowers in the larval stage. Within the genus in which multivoltinism and polyphagy prevail, *Celastrina sugitanii* Matsumura in Japan shows unique ecological traits of univoltinism associated with virtual monophagy (Fukuda *et al*., 1984). To understand how these traits have been acquired, some biological features of *C. sugitanii* were investigated in comparison with those of *C. argiolus* (Linnaeus), a multivoltine and polyphagous congener.

Distinct from multivoltine species in which inclusive fitness should be evaluated as annually accumulative or as an average value, univoltine species in every generation have to realize sound fitness in a restricted period under changeable environmental conditions. Predictable climatic variations in the temperate zone may continue to exercise selection pressure on ecological traits in univoltine species, and hence may develop some unique features in such species. Here we report rapid growth at lower temperatures in larvae of *C. sugitanii*, which should be an adaptation to cope with the shorter flowering period of the host tree, *Aesculus turbinata*. To synchronize the life cycle with food resources can be a significant norm not only in determining the diapausing period but also in the active period (Masaki, 1980).

Materials and methods

Field survey

To estimate the larval period in the field, numbers of adults and mature larvae falling from the host tree were counted in a population at Oshika-kei, Misasa-Cho, near Kurayoshi City,

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*Life cycles of the genus *Celastrina* in Japan, II

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Tottori Prefecture (alt. *ca* 640 m). Adult censuses were carried out from early April to mid May with intervals of several days, in 2003–2005, by two observers from 10: 00–16: 00. As the females tended to remain close to host trees, differing from the males, the censuses were performed around a host tree. Some adults were collected in order to obtain eggs for the following rearing test. To count falling mature larvae, we set large "litter-fall traps" under the host tree. The tree was 28 m in height, 1.55 m in trunk diameter and covered 389 m^2. Eight sheets for agricultural use were extended (127 m^2 in total area) and larvae trapped were collected every second day during May 24–28, 2004.

Rearing test

To examine oviposition preference, nine females collected from the above field were allowed to oviposit on flowers of *Aesculus turbinata* and/or *Swida controversa* for a given time in a cage (30 × 30 × 30 cm). Flower species offered were alternatively changed every hour or unchanged for several hours. Numbers of eggs laid were counted every hour. Eggs obtained in the above oviposition choice test were reared in plastic petri-dishes placed in incubators each set at 18, 21, 24, 27°C (all 16L8D), to determine developmental zero point and thermal constant (=effective cumulative temperature) for egg and larval period. Fresh flowers of *A. turbinata* were given intermittently as food, and hatch and molt were daily recorded. To compare with the values of developmental zero point and thermal constant obtained, the third and fourth generations of *C. argiolus* were also reared in August-September of the year under the same temperature/photoperiod condition but with flowers of *Pueraria lobata*.

Results

1. Oviposition preference

The results of oviposition choice test are given in Table 1 and show obvious preference for *Aesculus turbinata*. In the alternative test 101 eggs were laid on flowers of *A. turbinata* during 30 hours, while only one egg on those of *Swida controversa*. Even in the continuous test two out of five females rejected the latter completely. The values for *S. controversa* in the continuous test in Table 1, where 47 eggs were laid in 10 trials, might be overestimated due to one female, who laid 38 eggs in 5 trials and died the next day. Although females of the population examined have a physiological potential to utilize *S. controversa*, it should be seldom to realize under natural conditions. Indeed, we have never found any egg or larva of the butterfly on flowers of *S. controversa* in the habitat.

2. Larval period in the field, with notes on the falling larvae

Figure 1 depicts seasonal fluctuations in numbers of adults and falling larvae in 2004.

Table 1. Oviposition choice by female *Celastrina sugitanii* (Misasa population). Females allowed to contact the flower during one hour in each trial.

	Flower species offerred	No. of trials eggs laid/trials	No. eggs laid/trial* (mean±s.d.)	Females accepted
Alternative	*Aesculus turbinata*	13/30	7.77±7.42	5/5
	Swida controversa	1/30	1.00 –	1/5
Continuous	*Aesculus turbinata*	13/24	5.77±4.07	4/4
	Swida controversa	10/27	4.70±6.53	3/5
	Wisteria floribunda	0/8	– –	0/2

*trials not laid eggs are excluded.

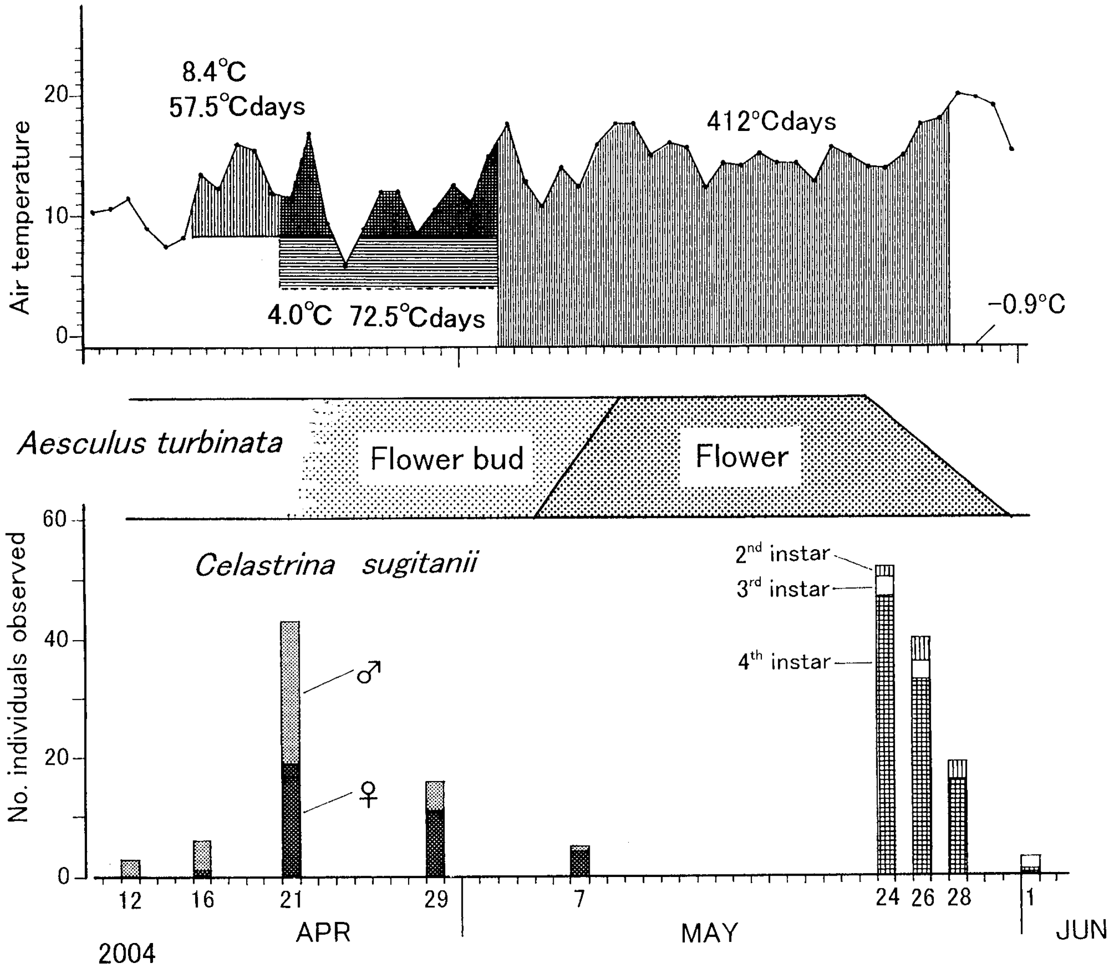

Fig. 1. Annual fluctuations in observed numbers of adults and mature larvae of *Celastrina sugitanii* in Oshikakei (alt. 640 m), Misasa, Tottori Prefecture, in 2004 (below). Flowering season of the host tree, *Aesculus turbinata*, and change in air temperature estimated are also given (above). Air temperature is estimated from that at Kurayoshi Meteorological Station (alt. 8 m) by reducing 3.6°C for altitudinal difference. See Table 3 and relevant text for meshed area of temperature.

Adults began to appear from mid April and ceased in early May with a peak on April 21 in this year. The adult appearance varied annually, however, and the peak was on April 28 and May 3 in 2003 and 2005, respectively. The peak of oviposition activity should be close to the peak presence of females, and was presumed to be on April 21 for 2004.

The larvae matured, at least most of them, fell from the canopy of the host tree *Aesculus turbinata ca* 25 m in height. Flowers of the host tree were at their best in mid May and fell in late May (Fig. 1). Although we are not sure whether the larva left the flowers spontaneously or fell passively together with falling flowers (so-called "parachuting"), a total of 114 larvae (96 of 4th instar, 9 of 3rd instar and 9 of 2nd instar) was collected by "litter-fall traps" mostly during May 24–28, 2004. Two out of 96 fourth instar larvae were parasitized by a parasitoid (unidentified). The fact that 94% (88/(96–2)) of the 4th instar larvae collected pupated within the next day, at midnight, suggests their spontaneous falling. The ratio also suggests a very low mortality due to the falling, despite that a falling speed could be estimated to be *ca* 9 m/s or 30 km/hr (referring to rain drops with 5mm diameter). The pres-

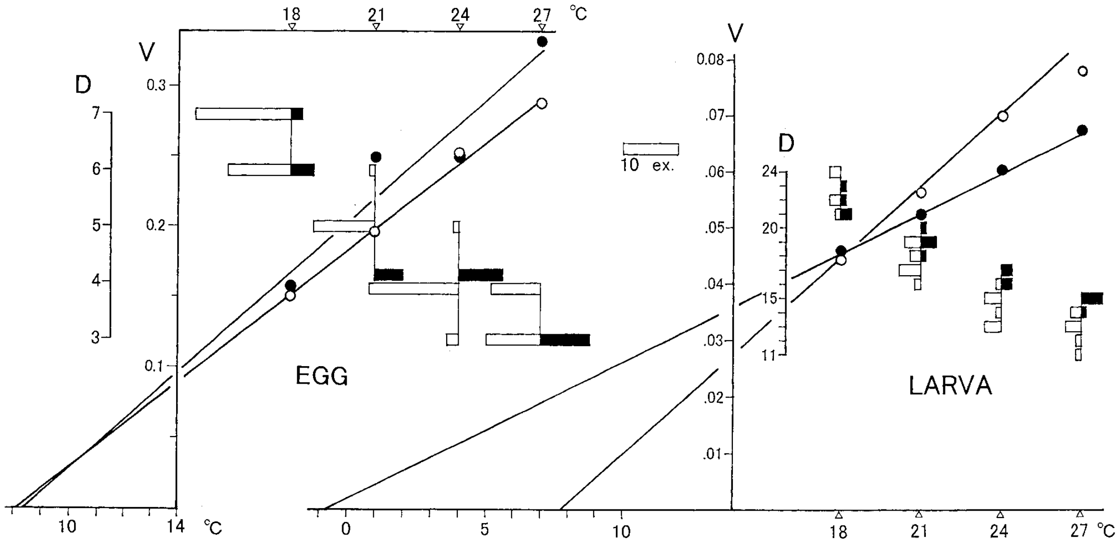

Fig. 2. Egg periods (left) and larval periods (right) in days (D) of *Celastrina sugitanii* (black bar) and *C. argiolus* (open bar) under various temperatures (abscissa). Regression lines of developmental rate (V=1/days) in relative to temperatures are shown with mean V values for *C. sugitanii* (black circle) and those for *C. argiolus* (open circle). Regression of larval period of *C. argiolus* was calculated using three V-values, as slightly arrested growth was observed in 27°C.

Table 2. Egg period and larval period of *Celastrina* butterflies in days under various temperature (°C). Photoperiod=16L8D. Given in mean±sd (N). Regression formulae are given below with correlation coefficient in parenthesis. V=1/days.

Stage	Temp. (°C)	*C. sugitanii* reared with *Aesculus turbinata*	*C. argiolus* reared with *Pueraria lobata*
Egg	18	6.33±0.52 (6)	6.61±0.50 (28)
(days)	21	4.00±0.00 (5)	5.08±0.29 (12)
	24	4.00±0.00 (8)	3.95±0.40 (19)
	27	3.00±0.00 (9)	3.47±0.51 (19)
Larva	18	21.75±0.96 (4)	22.60±1.34 (5)
(days)	21	19.00±0.71 (5)	17.70±1.06 (10)
	24	16.50±0.58 (4)**	14.25±1.16 (8)
	27	14.80±0.45 (5)	12.86±1.07 (7)
Regressions			
Egg		V=0.0175T–0.1469 (0.948)	V=0.0155T–0.1273 (0.996)
Larva		V=0.0024T+0.0021 (0.999)	V=0.0047T–0.0428 (1.000)*

*excluding a value of 27°C due to slightly arrested growth.
**two individuals emerged without diapause.

ence of 18 immature larvae simultaneously collected, however, indicates that a part of the larvae at least fell passively. Ten of the 18 pupated later, but 4 of the 3rd instar and 4 of the 2nd instar died under rearing, of which one and 4 larvae were parasitized, respectively. Based on these observations, we estimated that the peak of pupation in the field population should be on May 27 in the year.

These estimated peaks indicate that most of *C. sugitanii* passed their egg and larval stages within 36 days (April 21–May 27) in the field conditions of 2004.

Table 3. A comparison of developmental zero points (T_0; °C) and corresponding thermal constant (K; °C-day) between *Celastrina sugitanii* and *C. argiolus*. Calculated from data given in Table 2. Confidence limit of T_0 values (95%)* are in parenthesis.

	Egg		Larva	
	T_0	K	T_0	K
C. sugitanii	8.38 (3.96/12.14)	57.5 (72.5/42.5)	–0.88 (–1.48/–0.30)	412.4 (423.2/401.9)
C. argiolus	8.19 (7.17/9.22)	64.4 (69.3/59.5)	7.82 (6.62/8.95)	235.2 (254.6/211.7)

*temperatures at which upper/lower limits of regression (95% of confidence) across the axis V=0.

3. Effect of temperature on egg and larval growth

The periods of egg and larval stage under various temperatures are shown in Fig. 2 and values obtained are compiled in Table 2, with a reference to the closely related *C. argiolus*. For egg period, though the observation interval of one day was coarse for the period of 3–7 days observed, both insects showed similar responses to the temperatures to which they were exposed. Developmental zero points were *ca* 8.3°C, and thermal constants were *ca* 60°C-day in both species, however, the confidence range of the developmental zero point was very wide in *C. sugitanii* (Table 3).

Developmental zero point for larval period was obviously different between *C. sugitanii* and *C. argiolus* (Table 3). Although the latter showed a developmental zero point of 7.8°C, a value similar to that of the egg period (calculating from periods at 18, 21, 24°C since slightly arrested growth was observed at 27°C), the former revealed an extraordinary developmental zero point of –0.9°C. Despite that *C. sugitanii* spent larval periods 1.3–2.2 days longer than *C. argiolus* at 21, 24, and 27°C, while at 18°C the larval period of *C. sugitanii* was 0.85 days shorter than that of *C. argiolus* (Fig. 2, Table 2). No arrested growth was detected in *C. sugitanii* even at 27°C, revealing a good linearity in regression (Fig. 2). In *C. sugitanii*, because of the low developmental zero point, the value of the thermal constant for larval development became larger, being *ca* 410°C-day (Table 3).

Discussion

The extreme value of developmental zero point in *C. sugitanii* described above may or may not be caused by arrested growth in a wide range of higher temperatures above 21°C, since the larvae of the species grow under lower temperatures in spring. This view, however, can not explain the annual fluctuation observed in the field (Fig. 1). If larvae of *C. sugitanii* grew at the same rate as *C. argiolus*, they should spend more than 39 days as larvae under the field temperature conditions given in Fig. 1, and the females would have to oviposit in March! This is obviously not the case. Adopting the values of developmental zero point and thermal constant for *C. sugitanii* obtained in the present study (Table 3), the egg and larval periods can be predicted as 16 days and 26 days in the field, respectively; eggs laid on April 16 should hatch on May 2, and pupation takes place on May 27 (Fig. 1). This phenological reconstruction should be corrected in the egg period since developmental zero point for the period was not well determined (Table 3). Presuming the developmental zero for eggs as 4°C and the corresponding thermal constant as 72.5°C-day, the egg period is expected to be 11 days, hence for individuals that pupate on May 27 oviposition should have taken place on April 21, which coincides well with the field observation shown in Fig. 1, below.

The value of developmental zero point, –0.9°C, in larvae of *C. sugitanii* is unusual within butterflies (Bryant *et al.*, 1999; Kato, 2005) or even in whole insects (*cf.* Kiritani, 1997, 2001; Ito, 1975). In a monograph compiling developmental zero point of 430 insects,

Kiritani (1997) described the mean value of 83 lepidopteran species as 10.4°C±2.4 s.d. So far as we are aware, developmental zero point at subzero temperatures is hitherto known in aphids, *Macrosiphum euphorbiae* in Canada (–0.03°C: Rarlow, 1962) and *Aphis gossypii* in Japan (–0.4°C: Komazaki, 1982), and also a dipteran species, *Delia floralis* (–2.4°C: cited in Kiritani, 1997). The present case in a *Celastrina* butterfly may or may not be biased since it is estimated by a large extension of the regression line concerned (Fig. 2). Confidence limit lines of the regression (95%), however, cross an X-axis of V=0 at –1.48°C for the upper limit line and -0.30°C for the lower limit line. Data given by the present study expect that for *C. sugitanii* a larval period of 26 days at 15°C, 32 days at 12°C and 42 days at 9°C. A future experiment examining larval periods under such lower temperatures will provide crucial evidence. Irrespective of the absolute value of the developmental zero, rapid growth by larvae of *C. sugitanii* at lower temperatures should be true. We believe that in *C. sugitanii* the distinct adaptation has been acquired to utilize the seasonally restricted flower resource of *Aesculus turbinata*. Due to their univoltine life cycle with a long diapausing period, *C. sugitanii* in Japan have to accomplish almost all food assimilation within the restricted flowering period of *Aesculus turbinata* in May (Hoshikawa and Komeyama, 2007).

C. sugitanii might be bivoltine in the Korean Peninsula (Wakabayashi, *unpubl.*; see Fukuda *et al.*, 1984), and some non-diapausing pupae were also observed in the present study; out of 4 individuals reared at 24°C, 2 adults emerged without diapause (Table 2) and 3 adults emerged from 12 stock cultures reared under room temperature (24–27°C). Although such was never observed at other temperatures (Table 2), this exceptional phenomenon may suggest the occurrence of non-diapause gene(s) in the populations of Japan. The species may be strengthening (or have strengthened) the univoltine/ monophagous trend within the Japanese Archipelago. Several local populations of the butterfly utilize *Swida controversa* and *Phellodendron amurense* as subsidiary hosts (Fukuda *et al.*, 1984), and utilization of other leguminosid hosts in some fields was recently reported (Iwano *et al.*, 2006). To understand the process of host shift in the butterfly, seasonal restriction in resource materials or climatic adaptation of the species should be of significance. Geographic variation in the flowering period of *Aesculus turbinata* could also strongly affect the life cycle of the butterfly. We are now interested in the host utilization by populations in Hokkaido, the northern boundary of the species; whether or not the flowering period of *A. turbinata* in southern Hokkaido is sufficiently long to breed the larvae of *C. sugitanii*.

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摘　　要

スギタニルリシジミ幼虫の低温下での速い成長 (米山沙希・星川和夫)

山陰地方のスギタニルリシジミはトチノキのみに依存しているが, 本種の生活史を解析する目的で野外調査と室内飼育を行った. 成虫の発生消長から野外での産卵時期は4月下旬と推定され, 樹上から落下する成熟幼虫のリタートラップでの採集量の変化から, 幼虫がトチノキを離れるのは5月下旬と計測された. 採集された成熟幼虫のほとんどは直後に蛹化した. これらから調査地 (小鹿渓; 三朝; 鳥取県中部) での本種の卵・幼虫期間は約36日と推定された.

異なる温度条件 (18, 21, 24, 27°C; いずれも16L8D) でトチノキ花を与えて本種幼虫を飼育したところ, その発育零点は–0.9°Cと極端に低い値となり, そのため発育有効温量は412日度と大きな値となった. この発育零点は昆虫類全体の中でも極めて低い値であるが, 室内飼育実験で得られた温度発育速度回帰から野外調査地における幼虫期間を予測すると約26日となり (当該期間の平均気温は約15°C), 11日間の卵期間を考慮すれば, 野外調査に基づく上記推定期間とよく一致した.

同条件でクズを用いて飼育したルリシジミ幼虫の発育零点は7.8°Cだったので, スギタニルリシジミは特異的に低温域における速い幼虫発育を発達させたことが示唆される. これはまだ低温の春のトチノキの限られた開花期間中に, ほとんど全ての同化代謝を行わなければならない本種における著しい生活史適応のひとつと考えられる.

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